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Cadmium Bioaccumulation Assays. Their Relationship to Various Ionic Equilibria in Lake Superior Water

John E. Poldoski

U.S. Environmental Protection Agency, Environmental Research Laboratory—Duluth, 6201 Congdon Boulevard, Duluth, Minn. 55804

■ The potential use of bioaccumulation by *Daphnia magna* as an analytical tool in studying properties of various aqueous cadmium forms is described. Selected aquatic chemical factors affecting cadmium residues were determined for organisms exposed to cadmium in the absence and presence of various organic and inorganic chemicals, some capable of strongly complexing cadmium. Without any added complexing agents, steady-state relationships were observed between total aqueous cadmium concentrations (2×10^{-10}–9×10^{-8} M) and bioaccumulated cadmium (0.09–3.2 μg/g, wet weight) within 2–4 days of exposure. Residues were a nonlinear function of the concentration. The presence of humic acid, pyrophosphate, or aminopolycarboxylic acids, at sufficient concentrations to maximize complexation, was effective—to various degrees—in reducing cadmium uptake. However, cadmium in the presence of diethyldithiocarbamate bioaccumulated to a greater degree than in its absence.

Of several heavy metal pollutants in the aquatic environment, cadmium concentrations in some anthropogenic deposits have shown one of the largest relative increases, compared to natural conditions (*1*). In addition, reported cadmium concentrations in natural waters can show more than two orders of magnitude variation (*2, 3*). Although the need for determining concentrations of the free metal, $Cd(H_2O)_x^{2+}$, is presently becoming recognized (*4, 5*), it is also conceivable that knowledge of other metal forms may be equally important (*6*).

Because of the need for different types of chemical measurements, which help to reflect the biological significance of various chemical species present in an aquatic environment, studies were undertaken to determine the feasibility of using short-term bioaccumulation as an analytical tool. *Daphnia magna* was chosen for investigation since it is commonly used as a bioassay test organism, and its bioaccumulation rate was initially found to be rapid. This paper explores some possible analytical relationships between ionic chemical equilibria for cadmium in Lake Superior water and the ability of daphnids to concentrate cadmium from some different aqueous chemical environments.

Data will be presented showing the resultant cadmium body burden as a function of exposure time, cadmium concentration, the complexing agent, and major cations. The following complexing agents were studied: a commercial grade humic acid (HA), pyrophosphate (P_2O_7), nitrilotriacetic acid (NTA), diethyldithiocarbamate (DDC), ethylenediaminetetraacetic acid (EDTA), and ethylenebis[*N,N'*-(2,6-dicarboxy)]piperidine (EBDP), a structural analogue of EDTA.

Experimental

Apparatus. Measurements of total cadmium, copper, nickel, zinc, lead, and chromium were made by computer-assisted (*7*) furnace atomic absorption spectrometry (FAAS). Instrumentation (Perkin-Elmer) included a Model 305B atomic absorption spectrophotometer equipped with a deuterium arc background corrector, a Model 56 recorder, and a Model AS-1 autosampler with Teflon (TFE) sample cups. Instrumental settings that were used have been given in previous reports (*2, 8*), and graphite tubes were coated with pyrolytic graphite similar to that described by Manning and Ediger (*9*).

An Orion Model 801 pH meter, equipped with a thermostated (15.0 °C) 50-mL FEP Teflon cell containing a cadmium ion selective electrode (Orion 9448A) and a double junction reference electrode (Orion 900200), was employed for potentiometric measurements of free cadmium (ISE).

For determinations of labile cadmium by anodic stripping voltametry (ASV), a modified Heathkit module (*10*) equipped with a Hewlett Packard Model 2D-2 X–Y recorder and a wax-impregnated graphite mercury film electrode was employed. Procedural details, such as preparation of the working electrode, are the same as described elsewhere (*11*), except no mercury(II) was added to samples or standards analyzed under the conditions of this study.

Measurements by differential pulse polarography were made with a Parr Model 174 equipped with a Hewlett Packard Model 2D-2 X–Y recorder and a 50-mL FEP Teflon cell containing a dropping mercury electrode, a platinum auxiliary electrode, and a Ag–AgCl (0.1 M NaCl) reference electrode.

Reagents. EBDP was prepared in the laboratory (*12*); however, other chemicals were obtained from commercial ACS

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reagent grade sources. Humic acid was technical grade (H1675-2, Aldrich). Stock solutions of complexing agents were prepared by dissolving the acid form or the sodium salt in deionized, distilled water (Millipore, Super Q System), with pH adjustments made with sodium hydroxide or sulfuric acid. Nitric acid (Baker Ultrex) was used for tissue digestions and acidifying solutions for cadmium analysis. Standard cadmium (8.9×10^{-3} M) stock solutions (pH 3), used for daphnia tests and measurements of free cadmium, were prepared by weighing the sulfate salt, and atomic absorption standard stock solutions (8.9×10^{-3} M) were prepared by weighing cadmium metal (99.99% purity). The prepared standards agreed to within ±5% of stated values for trace metal reference samples for water issued by the U.S. Environmental Protection Agency, EMSL—Cincinnati.

General Procedure. Daphnia were obtained from a clone maintained at the Environmental Research Laboratory—Duluth. The organisms were cultured and tested using procedures previously described by Biesinger and Christensen (*13*), except where noted otherwise. Lake Superior test solutions (200 mL) and six average-size daphnid adults were placed in cleaned 250-mL Pyrex beakers with no food added. Test solutions were prepared by adding the required aliquot of cadmium to Lake Superior water (water was obtained from the Environmental Research Laboratory—Duluth (*13*)) followed by the addition of any other chemicals as required. A 5-min equilibration time was allowed prior to exposure to daphnia. Each set of tests (15 ± 1 or 20 ± 2 °C) was comprised of a control (Lake Superior water) and reference exposures (usually in duplicate or triplicate) with 8.9×10^{-8} M added cadmium. Test exposures were generally prepared in at least duplicate, and resultant residues were compared to those obtained for the reference exposure. At the end of each test, a sample of water and a composite of four to six live daphnids per exposure were routinely measured for total cadmium by FAAS, as subsequently described. On selected water samples, pH, specific conductance, labile cadmium, and free cadmium were also measured.

Daphnids were prepared for residue analysis by transferring approximately 5–10 mg of sample (6 daphnids) with a transfer pipet from the exposure water to a 0.45-μm Millipore filter. They were liberally rinsed with deionized distilled water and, along with a stream of dry nitrogen directed at the animals, vacuum was applied to the filter apparatus for 0.5 min to draw off excess water. Immediately after (<1 min), a clean Teflon micro-spatula was used to dislodge each daphnid from the filter to a precleaned and tared polypropylene weighing boat, which was then weighed to the nearest 10 μg. The composite sample was transferred to a decontaminated 1.5-mL capacity cone-shaped TFE sample cup, specifically prepared for micro-size sample digestions. Known amounts of cadmium were occasionally added to control samples at this point to check and assure that the recovery was near 100%. Nitric acid (0.1 mL) was added to each sample cup—including 2–3 digestion blanks per test—and heated in a water bath for 0.5 h at approximately 95 °C. The digests were diluted with 1 mL of deionized distilled water and stored at 4 °C until analysis, which was usually performed on the same day. All results were expressed on a wet-weight basis.

Routine FAAS analysis of cadmium in digests involved making reagent additions to both standards and digests so that they would contain approximately 0.15% w/w lanthanum and 2% v/v nitric acid. Calibration was obtained by using the method of standard additions to digests of daphnia control samples.

Free cadmium was measured by ISE in water samples where daphnids were exposed, both in the absence and presence of the complexing agents, pyrophosphate or humic acid. Measurements were made similar to that previously stated (*2*) by adding known concentrations ($9–90 \times 10^{-7}$ M) of cadmium to samples (pH 7.5) and comparing resultant potentials to a standard curve prepared in a noncomplexing borate-buffered (pH 7.5) media. The ratio of free cadmium to total added cadmium was determined to be constant over a tenfold concentration change as observed in previous work (*2*). Therefore, a ratio determined at a higher concentration was assumed to be similar in the original sample containing a lower concentration. This ratio was then used to estimate free cadimum at total cadmium concentrations of approximately 9×10^{-8} M and lower.

Labile cadmium in the raw Lake Superior samples containing either NTA, EDTA, or EBDP was measured by ASV. Using considerations similar to that described in a study of the EDTA complex (*14*), labile cadmium was estimated and reported as free cadmium in this paper. Briefly, a 10-mL aliquot of sample was placed in a quartz cell and degassed with a water-saturated N_2–CO_2 gas mixture (3–5 min) until a stable pH (6.4 ± 0.1) could be maintained. This pH was chosen because of the greater difficulty in adjusting the gas mixture to maintain pH 7.5 conditions. Plating (2 min) was performed at −1.0 V and the potential was scanned linearly from −1.0 to −0.4 V at a rate of 10 V/min. Peak heights for the calibration curve were linear with concentration in the range of $0.05–9 \times 10^{-8}$ M and corresponded to labile cadmium ion. Peak heights obtained for samples containing the above-mentioned complexing agents were used to calculate the labile cadmium concentration (pH 6.4) from the calibration curve. Labile cadmium at pH 7.5 was obtained by multiplying the pH 6.4 value by a calculated factor. This factor was the ratio of calculated free cadmium at pH 7.5 to that at pH 6.4 for each corresponding chemical system.

Analyses of DDC in test solutions were made by differential pulse polarography, similar to analyses previously reported for other reduced sulfur-containing compounds (*15*). Aliquots of sample (50 mL) containing $NaNO_3$ (0.02 M) were degassed with water-saturated nitrogen for 5 min prior to initiating the scan. Instrumental operating conditions were: 5 mV/s scan rate, −0.3 to −1.05 V scan range, 2-s drop time, 0.016-cm^2 drop area, and a 50-mV pulse amplitude. Well-defined peaks were obtained at −0.73 V, and unknowns were calculated by comparing their peak heights to a standard curve obtained for freshly prepared standards in Lake Superior water within the concentration range of 10^{-6}–10^{-5} M DDC.

Calculations. Equilibrium calculations were accomplished on a Hewlett Packard Model 3354 computer. A BASIC language program was written, specific for these chemical systems, to perform a simple iterative refinement procedure on mass balance equations using a 0.1% convergence criterion. Stability constants (*12, 16*) used to calculate free cadmium were corrected, using the Davies equation, to correspond to the approximate ionic strength of the test media (the ionic strength of Lake Superior water is about 0.001).

The acid stability constant for DDC was not precisely known; however, its proton should be no less acidic than those associated with thiol functional groups in compounds such as cysteine. Therefore, it was estimated that an acid stability constant of 10^8 was a reasonable approximation, and this value was used in calculating free cadmium concentrations with DDC as a ligand. Furthermore, since all experiments were performed at pH 7.5, it follows that any error in estimating this constant had only a minor effect on the results. Thus, it was not of major importance because the intent of these calculations was to only show that most of the cadmium was complexed.

Complexes with hydroxide, sulfate, carbonate, and chloride ions were considered, but they generally formed to small or negligible degrees, except only under a few of the experimental conditions.

Table I. Typical Analytical Reproducibility for Duplicate Residue[a] Determinations

sample no.	sample wt, mg	residue found,[b] μg/g anal. A	residue found,[b] μg/g anal. B	ratio A/B
1	2.45	3.2	2.7	1.18
2	2.25	3.8	3.4	1.11
3	2.88	3.1	2.9	1.07
4	6.43	3.1	2.9	1.06
5	8.06	3.4	3.6	0.94
6	7.58	3.6	3.5	1.03
7	17.17	2.8	2.9	0.96
8	15.50	2.7	2.7	1.00
9	16.11	2.9	3.0	0.97

means: A/B = 1.04 ± 0.08

A = 3.2 ± 0.4 μg/g

B = 3.1 ± 0.3 μg/g

no. 1–3 = 3.2 ± 0.1 μg/g

no. 4–6 = 3.4 ± 0.3 μg/g

no. 7–9 = 2.8 ± 0.1 μg/g

[a] All daphnids exposed to 8.9×10^{-8} M cadmium in Lake Superior water. [b] Analyses A and B are two independent analytical runs performed on the same digests. ± indicates standard deviation. Values correspond to wet weight.

Table II. Effect of Sample Size and Lanthanum Addition on Analysis Spike Recovery of Cadmium

added La, % w/w	% recovery: 4 mg of sample/mL of digest	% recovery: 1 mg of sample/mL of digest
0	57	82
0.025	55	88
0.05	87	95
0.15	94	98
0.40	95	

Table III. Base-Line Trace Element Concentrations in Composite Samples of Daphnids Used as Controls

element	mean residue, μg/g	no. of determ.
Cd	0.09 ± 0.04	49
Pb	0.06 ± 0.03	2
Cu	3 ± 2	2
Zn	5 ± 0.4	2
Ni	<0.2	2
Cr	0.06 ± 0.02	2

Results and Discussion

In past studies, exposure of some aquatic organisms to water contaminated with cadmium has resulted in continual large increases in cadmium residue over long time periods (several months), as determined for fish exposed to less than 10^{-7} M cadmium concentrations in the water (*17*). In contrast, results of this work strongly indicated that bioaccumulation was dependent on the form of cadmium and that a relatively steady-state relationship between total aqueous cadmium and bioaccumulated cadmium generally occurred within a 2–4 day period of exposure. Secondly, the methodology used for residue analyses of daphnia suggested that these bioaccumulation assays can be attractive with respect to time considerations.

Residue Analysis. The precision of weighing daphnids (wet) and performing multiple residue analyses on samples of different size was first determined to help identify sources of error. As an example, replicate weighings of a single sample showed that weighing was a significant factor affecting overall reproducibility: 5.85, 5.23, 5.05, 5.54, and 6.35 mg wet weight (mean = 5.60 ± 0.51 mg). The 9% relative standard deviation was mostly attributed to the uncertainty of removing water from the daphnids. This compared to the reproducibility in the duplicate atomic absorption measurement of a single set of digests, as indicated in columns A and B of Table I. The mean of A/B (1.04 ± 0.08) demonstrated an analytical agreement of about 4%. In addition, results for three different sample weight ranges (no. 1–3, 4–6, and 7–9) showed relatively little difference in residues. Although drying the daphnids might have generally given less variability in weights, it would have also increased the total analysis time and the possibility of contamination.

One problem initially encountered was low and variable FAAS cadmium analysis recoveries for daphnia digests. Recoveries were sometimes lower than 50%, but they also depended on the concentration of sample in the digest. However, further study indicated that recoveries were essentially independent of the digest matrix and sensitivity was increased if lanthanum nitrate was added to the samples. This observation was similar to that reported by Andersson (*18*) for the analysis of lead. Since the addition of lanthanum made it possible to simplify the analysis, additional experiments were performed to determine its optimum concentration for the conditions of this study. Table II shows that increasing its added concentration from 0 to 0.40% w/w resulted in a recovery improvement from 57 to 95%. A value of 0.15% w/w lanthanum was chosen for routine use since higher concentrations resulted in no further relative improvement, but only increased the blank and reduced the lifetime of graphite tubes. For the water samples, a lower value of 0.05% w/w lanthanum was adequate.

Baseline Element Concentrations. Because it was possible that various other trace element concentrations in daphnia may affect cadmium residues, baseline analyses of control daphnia were performed for selected trace elements (Table III) to help define conditions under which experiments were performed. Except for nickel, residues for all the metals were detected using the digestion procedure given for cadmium. Insufficient data did not allow for a comparison to residues in daphnids from other natural freshwater systems. In addition, the major ionic composition (*13, 19*) and trace metal content (*8, 13*) reported for Lake Superior water indicated that the source of exposure waters was fairly stable and of relatively low concentration with respect to the metals.

Bioaccumulation of Free Cadmium. It can be readily calculated from equilibrium constants and the known major chemical characteristics of Lake Superior water that free cadmium should represent greater than 0.9 of the total fraction of dissolved cadmium. In support of this, ion selective electrode measurements of cadmium added to lake water—for concentrations as low as 9×10^{-8} M—indicated no detectable degree of complexation. In addition, the linear nature of ASV calibration curves ($0.05–9 \times 10^{-8}$ M) suggested undetectable nonlabile complex formation. Because these three determinations were internally consistent with each other and in accord with other work (*20*), it was assumed that free cadmium was the predominant species in tests without added complexing agents.

The data for mean residue vs. exposure time at several different cadmium concentrations are illustrated in Figure 1. Over periods of 0–4 days, water and daphnids were sampled and analyzed from each of a series of five different exposure

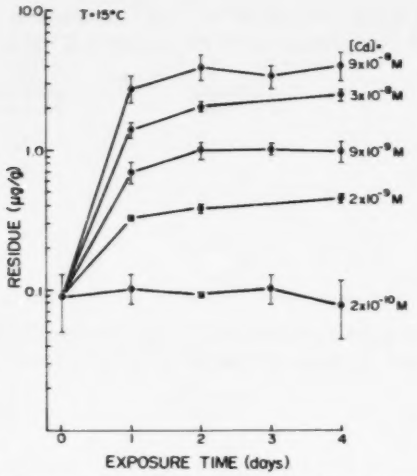

Figure 1. Effect of exposure time on cadmium residues. The 2×10^{-10} M cadmium concentration corresponds to the control value

Table IV. Correlation of Cadmium Residues in *Daphnia magna* with Various Cadmium Concentrations in Lake Superior Water

nominal added Cd, μM	mean measured total Cd, μM	mean residue ratio[a]	no. cases	calcd Cd[b] via Eq 1, μM
0	≦0.0002	0.023 ±0.008[c]	13	0.00001
0.0022	0.0021 ±0.0005	0.106 ±0.02	13	0.0026
0.0089	0.0085 ±0.0009	0.248 ±0.05	14	0.0082
0.0267	0.0268 ±0.0028	0.536 ±0.08	13	0.0269
0.0890	0.0921 ±0.0052	1	14	0.0921

[a] Residue ratio = test residue/mean reference residue for 2-day simultaneous exposures at 8.9×10^{-8} M cadmium. [b] Measured mean total cadmium concentration in water correlates to mean residue ratio via a cubic expression (Equation 1), with a multiple correlation coefficient = 0.9999 (on an individual basis, 67 cadmium (H_2O)–residue ratio data pairs correlate via a similar cubic expression giving a multiple correlation coefficient of 0.9848). [c] ± indicates standard deviation.

concentrations (2×10^{-10}–9×10^{-8} M at $T = 15$ °C). Since the 3-week LC_{50} and 4-day LC_{50} for *Daphnia magna* are about 5×10^{-8} and 6×10^{-7} M cadmium (*13*), respectively, these concentrations and exposure times represented essentially nonlethal conditions. A plot of mean residue (three–five determinations per data point) vs. exposure time clearly indicated a rapid uptake and a relatively steady state between the water and body concentrations. This characteristic of daphnids to reach a steady state with certain other chemicals has also been observed by different workers (*21, 22*). To investigate the effect of small changes in temperature, a series of experiments was conducted at both 15 and 20 °C. Within 2–3 days of exposure to 9×10^{-8} M cadmium, no consistent dependence on temperature was detected. An overall average (experiments at both 15 and 20 °C) for 76 exposures of 2–4 days duration at $8.9 \pm 0.6 \times 10^{-8}$ M cadmium was 3.2 ± 0.8 μg/g.

Since the natural process of daphnid growth involved molting or loss of the exoskeleton, it was possible that this factor could influence the observed cadmium residue as a function of time, as observed for other species (*23*). Thus, to assure comparability of results, each set of tests comparing the effect of added chemicals on uptake was performed simultaneous with control exposures and reference exposures at 8.9×10^{-8} M free cadmium. The increase or reduction of cadmium residue in the presence of an added chemical concentration, in combination with 8.9×10^{-8} M cadmium, was expressed as the ratio of the test residue to the mean of the residue for the reference exposures. As given in Table IV, this experimental design was employed for obtaining data and calculation of the dependence of observed residue on cadmium concentrations in water. It is apparent that the mean residue ratio (R) was not linear with the mean cadmium concentration (μM). However, a cubic polynomial of the form:

$$(Cd)_{H_2O} = -0.0007 + 0.0285R + 0.0179R^2 + 0.0464R^3 \quad (1)$$

fit the data, with a multiple correlation coefficient of 0.9999.

Effect of Added Chemicals. Relative changes of cadmium residues to induced changes in the chemical characteristics of Lake Superior water are displayed in Table V. In most cases, apparent and calculated free cadmium concentrations are given. Experimental evidence is also provided, in most cases, to show the degree of cadmium binding in the water phase. Furthermore, to investigate equilibration time, the rate of formation of the EDTA complex was followed with ASV by observing the stripping peak as a function of time. With 8.9×10^{-8} M cadmium in Lake Superior water, complex formation appeared complete within 2 min, since no stripping peak was observed after the addition of 10^{-6}–10^{-4} M EDTA.

For DDC, measurements of free cadmium were not reliable by either potentiometric or ASV measurements due to interference by the compound. Because of the response reported for certain sulfur compounds (*24*) using a solid-state ion selective electrode (metal–sulfide sensing element), quantitation of free cadmium was not performed. Rather, DDC was measured in test solutions by differential pulse polarography to assure that the compound was present in solution at a sufficiently high concentration to promote complete complexation.

Complexing agents (no. 1–6) represented a limited cross section of compounds with various chemical characteristics. Humic acid generally appeared to be a relatively weak binding agent for cadmium. This is consistent with other data (*20*), although it is also suggested that there may exist different strongly binding natural organic components in environmental samples (*6*). The aminopolycarboxylic acid chelators NTA, EBDP, and EDTA all form relatively strong complexes with cadmium as does DDC; however, some very marked chemical differences exist. Unlike all the other complexes, free cadmium reacts strongly with the water-soluble sodium salt of DDC to form a relatively symmetric 2:1 hydrophobic coordination compound. This is probably a result of saturation of both the charge and the coordinate valence of the cation in addition to a high degree of covalent bonding character (*25*). As a possible consequence of this character, there are observed marked differences with respect to the compound's characteristic to stimulate the bioaccumulation of cadmium. The residue ratio for DDC (no. 6) indicated an uptake rate and equilibrium in excess of that for just free cadmium ion; however, the presence of other complexing agents (no. 1–5) clearly reduced cadmium residues to varying degrees. These observations probably reflect a relatively greater lipophilic character of the DDC complex, and may imply involvement of a different pathway of bioaccumulation. It is conceivable that other metal complexes having similar chemical properties would stimulate accumulation in a like fashion. Such examples might be the fungicide, ethylenebis(dithiocarbamate), or the mining mill flotation agent, potassium amyl xanthate, both

Table V. Effect of Various Chemical Additions to Lake Superior Water on Cadmium Concentration Changes in *Daphnia magna*[a]

no.	added chem	added concn, $\times 10^8$ M	days exposed	residue[b] ratio	app[c] free Cd, $\times 10^8$ M	calcd[d] free Cd, M	measd free Cd, $\times 10^8$ M	measd total Cd, $\times 10^8$ M
1	HA	50[e] (147)[f]	1	0.61 ± 0.09 (2)[g]	3.4		5.2	8.4
			2	0.58 ± 0.08 (4)	3.0		3.3 ± 0.6	7.8
			3	0.47 ± 0.08 (2)	2.1		3.7 ± 0.3	7.5
2	$Na_4P_2O_7$	500 (165)	1	0.13 ± 0.01 (2)	0.4	2×10^{-10} (2×10^1)[h]	0.027	9.6
			2	0.13 ± 0.04 (4)	0.4	2×10^{-10} (2×10^1)	0.051	8.8
3	NTA	100 (71)	2	0.35 ± 0.07 (4)	1.3	1×10^{-10} (1×10^2)	<0.01	9.7
		1 (71)	2	0.57 ± 0.09 (4)	3.0	1×10^{-8} (3)	1.1	8.5
4	EBDP	100 (70)	2	0.06 ± 0.01 (4)	0.1	1×10^{-13} (1×10^4)	<0.01	9.6
5	EDTA	100 (71)	2	0.10 ± 0.02 (3)	0.3	3×10^{-13} (1×10^4)	<0.07	9.4
		1 (71)	2	0.11 ± 0.02 (4)	0.3	6×10^{-11} (5×10^1)	<0.04	10.5
6	DDC[i]	50 (74)	1	0.94 ± 0.18 (2)	8.0	1×10^{-26} (1×10^{19})		6.4
			2	1.56 ± 0.34 (2)	27	1×10^{-26} (3×10^{19})		3.0
		10 (74)	2	1.46 ± 0.47 (3)	22	2×10^{-25} (1×10^{18})		5.3
7	Na_2SO_4	870 (149)	2	1.12 ± 0.12 (3)	12	8×10^{-8} (1.5)	>8	8.7
		4347 (468)	2	1.14 ± 0.10 (4)	12	6×10^{-8} (2.0)		8.4
8	NaCl	4347	2	0.99 ± 0.19 (3)	8.9	1×10^{-8} (1.3)		9.5
9	$CaSO_4$	1250 (295)	2	0.67 ± 0.06 (4)	4.0	8×10^{-8} (0.5)		9.1
		3750 (890)	2	0.61 ± 0.05 (4)	3.4	6×10^{-8} (0.6)	>8	9.9
10	$Ca(NO_3)_2$	3750 (691)	2	0.58 ± 0.08 (4)	3.1	9×10^{-8} (0.3)	>8	9.2

[a] Conditions: added cadmium = 8.9×10^{-8} M, pH 7.5 ± 0.2. [b] Residue ratio = test residue/mean reference residue for simultaneous exposures at 8.9×10^{-8} M cadmium with same exposure time. [c] Calculated from the observed residue ratio and assuming Equation 1. [d] Calculated using equilibrium constants. [e] Concentration units are mg/L. [f] Parentheses indicate specific conductance (μmho/cm, T = 15 °C). [g] Parentheses indicate number of determinations. [h] Parentheses indicate ratio of apparent free cadmium/calculated free cadmium. [i] Daphnids exhibited apparent mortality in the presence of this compound and cadmium

forming relatively hydrophobic metal complexes.

Proposal of a detailed mechanism of bioaccumulation is beyond the scope of this work; however, one of the commonly envisioned pathways of metal ion uptake by aquatic organisms involves an initial binding of the metal to form an ion pair or complex immobilized near a membrane surface. Although this may be a possible general route, it appears that the relation of certain ionic equilibria to partitioning of various organometallics into the lipid layer portions of membrane structure may also be important. Furthermore, it is conceivable that the ability of cadmium to displace another metal ion (e.g., zinc) from a binding site within daphnia may be aided or hindered by a complexing agent, depending on the type present (e.g., DDC vs. EDTA). For example, comparisons of the apparent cadmium concentrations in water, obtained by using measured mean residue ratios in Equation 1, to both calculated and measured free cadmium values are revealing. Even though the calculated and measured free values are in reasonably good agreement, they differ considerably from corresponding apparent values, for P_2O_7, NTA, EBDP, and EDTA (no. 2–5). Although calculated free cadmium concentrations with 10^{-4} M concentrations of these compounds are near or below the value for the controls (2×10^{-10} M), it is clear that the accumulation of cadmium is not completely suppressed. Based on the residues, the apparent free cadmium concentrations range from 1×10^{-9} to 1.3×10^{-8} M. On the other hand, agreement is fairly close for humic acid, with differences between measured and apparent values being less than a factor of three. Of all the compounds tested, probably the greatest relative distance can be seen between the calculated value of 1×10^{-26}

Table VI. Effect of Filtration and Complex Type on Total Cadmium Concentrations[a]

filter pore size, μm	measured total cadmium, $\times 10^8$ M			
	no added complexing agent	P_2O_7, 5×10^{-4} M	HA, 50 mg/L	DDC, 5×10^{-5} M
unfiltered	9.0	8.9	9.1	7.8
0.45	8.1	8.9	8.5	7.1
0.10	8.0	8.9	5.2	1.8
0.05	8.2	8.8	4.1	1.0

[a] Spiked Lake Superior samples aged 1 day at 20 °C and pH 7.5 prior to filtration. Nominal total added cadmium was 8.9×10^{-8} M.

M and the apparent value of 2.7×10^{-7} M for the presence of 5×10^{-5} M DDC, as indicated by their ratio given in Table V. These data indicate that cadmium accumulation in *Daphnia magna* is clearly not just a simple function of complexed vs. free cadmium in the aqueous phase, but it is strongly dependent on the type of complexing agent present.

Since calcium can comprise one of the major hardness components of natural water and may affect toxicity (*26*), several exposures of daphnids to cadmium were performed with added calcium salts in Lake Superior water to determine their effects on the short-term bioaccumulation of cadmium. The effect from both calcium and sodium salts is given in Table V (no. 7–10). Base-line calcium and sodium concentrations in Lake Superior water are approximately 3.2×10^{-4} and 4.8×10^{-5} M, respectively. It is seen that raising the total calcium concentration five times above the base-line value resulted in approximately one-third lower cadmium uptake. Therefore, in accordance with toxicity studies, hardness components in natural water appear to be factors to consider in interpreting these bioaccumulation data. Sodium ion concentrations 100-fold above base-line concentrations, added as either the sulfate or chloride salt, appeared to effect little change in residues, even though calculated free cadmium concentrations were slightly less than the total.

Differences in "dissolved" vs. particulate phases were considered (Table VI) as a function of filter pore size and the absence or presence of complexing agents. With progressively smaller pore sizes, the cadmium concentration in the filtrate showed a sharp drop at a 0.1-μm pore size and below in the presence of DDC or HA. For HA, the total "dissolved" cadmium appeared slightly greater than the apparent free cadmium and measured free cadmium as given in Table V. Thus, a large portion of the bound cadmium may be filtered from solution for this particular source of natural organic material. For DDC, 90% or more of the total added cadmium may exist as a hydrophobic form that does not pass the filter.

Summary

These data show steady-state bioaccumulation of cadmium by daphnia within 2–4 days and exemplify some types of chemicals affecting the degree of bioaccumulation. The addition of cadmium complexing agents to the system can markedly increase or decrease metal uptake by the organism. This has significance, particularly in light of data (*17*) supporting that cadmium has a long biological half-life for release from tissues. It appears that the use of daphnids as an analytical tool for rapidly determining "biologically available" cadmium in different chemical environments holds some promise. Future studies will deal with other chemical systems.

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16. ABSTRACT

The potential use of bioaccumulation by Daphnia magna as an analytical tool in studying properties of various aqueous cadmium forms is described. Selected aquatic chemical factors affecting cadmium residues were determined for organisms exposed to cadmium in the absence and presence of various organic and inorganic chemicals, some capable of strongly complexing cadmium. Without any added complexing agents, steady-state relationships were observed between total aqueous cadmium concentrations (2×10^{-10}-9×10^{-8} M) and bioaccumulated cadmium (0.09-3.2 µg/g, wet weight) within 2-4 days of exposure. Residues were a nonlinear function of the concentration. The presence of humic acid, pyrophosphate, or aminopolycarboxylic acids, at sufficient concentrations to maximize complexation, was effective--to various degrees--in reducing cadmium uptake. However, cadmium in the presence of diethyldithiocarbamate bioaccumulated to a greater degree than in its absence.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
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